SSSS had positive anti-Dsg1 IgG by ELISA as well as immunoblotting, although serum was obtained only at day 6 (SSSS no. 6 in Table 2). No anti-Dsg3 IgG was detected either by ELISA or by immunoblotting in these patients studied throughout their course, indicating the specificity of the reactivity against Dsg1 (Table 1). No apparent IgM reactivity against Dsg1 or Dsg3 was detected by ELISA (data not shown). None of the 12 patients with BI, whose sera were taken with a range of days 2–33, showed any detectable IgG production against either Dsg1 or Dsg3 as determined by ELISA and immunoblotting (Table 1).

These findings indicate that a small number of patients of SSSS develop low titers of IgG antibodies specific for Dsg1 after binding and systemic digestion of Dsg1 by staphylococcal ETs. This observation is specific for patients with SSSS because none of the patients with BI showed any sign of anti-Dsg1 antibody production. This may be because a single episode of BI may be too limited to cause an immune response. Although none of these patients develop PF after SSSS, which is not known as a predisposing factor for PF, the findings presented here provide evidence that infection which modifies self-antigen can trigger the production of IgG autoantibodies against the self-antigen. Of course, these patients do not develop clinically overt PF. We would speculate that other genetic or environmental factors are needed to extend the immune response to encompass pathogenic antibodies and to produce overt clinical PF. For example, in the endemic form of PF, fogo selvagem, that is found in rural areas of South America, it might be possible that repeated modification of Dsg1 by chronic or recurrent staphylococcal infection in genetically susceptible individuals might trigger disease.

Thus, our data are consistent with the hypothesis that a bacterial toxin can modify a self-antigen to result in an autoantibody response. The relevance of this finding to onset of autoimmune diseases remains to be proven.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Dr Takeji Nishikawa for insightful discussion and dermatologists in affiliated hospitals of Keio University School of Medicine for the collection of serum samples, and Ms Minae Suzuki and Ms Yoshiko Fujii for technical assistance. This work was supported by Health and Labour Sciences Research grants for Research on Measures for Intractable Diseases, from the Ministry of Health, Labor, and Welfare, and by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, of Japan, and Grants from the National Institute of Health.

Hidemi Anzai[1], John R. Stanley[2], and Masayuki Amagai[1]

[1]Department of Dermatology, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan and [2]Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA.
E-mail: amagai@sc.itc.keio.ac.jp

REFERENCES

Amagai M, Komai A, Hashimoto T, Shirakata Y, Hashimoto K, Yamada T *et al.* (1999) Usefulness of enzyme-linked immunosorbent assay (ELISA) using recombinant desmogleins 1 and 3 for serodiagnosis of pemphigus. *Br J Dermatol* 140:351–7

Amagai M, Matsuyoshi N, Wang ZH, Andl C, Stanley JR (2000) Toxin in bullous impetigo and staphylococcal scalded skin syndrome targets desmoglein 1. *Nat Med* 6:1275–7

Green KJ, Gaudry CA (2000) Are desmosomes more than tethers for intermediate filaments? *Nat Rev Mol Cell Biol* 1:208–16

Melish ME, Glasgow LA (1970) The staphylococcal scalded skin syndrome: development of an experimental model. *N Engl J Med* 282:1114–9

Payne AS, Hanakawa Y, Amagai M, Stanley JR (2004) Desmosomes and diseases: pemphigus and bullous impetigo. *Curr Opin Cell Biol* 16:536–43

Wucherpfennig KW (2001) Mechanisms for the induction of autoimmunity by infectious agents. *J Clin Invest* 108:1097–104

Both Pimecrolimus and Corticosteroids Deplete Plasmacytoid Dendritic Cells in Patients with Atopic Dermatitis

Journal of Investigative Dermatology (2006) **126,** 2141–2144. doi:10.1038/sj.jid.5700368; published online 11 May 2006

TO THE EDITOR

Atopic dermatitis (AD) is one of the most common inflammatory skin disorders characterized by pruritus, a chronically relapsing course and typically distributed lesions with dry skin, excoriations, and lichenification (Leung *et al.*, 2004). Topical treatment of acute lesions with corticosteroids (CS) is a mainstay in the therapy of the AD; however, long-term application is limited by CS-related side effects (e.g., skin atrophy) (Stoppolino *et al.*, 1983). The calcineurin inhibitors tacrolimus and pimecrolimus are now available as ointment/cream preparations and have proven to be a novel option for the topical treatment of AD (Ruzicka *et al.*, 1997; Luger *et al.*, 2001).

AD skin lesions harbor a variety of inflammatory cells, of which dendritic cells (DCs) represent a major fraction. Besides resident Langerhans cells and inflammatory dendritic epidermal cells,

Abbreviations: AD, atopic dermatitis; BMV, beta-methasone-17-valerate; CS, corticosteroids; DC, dendritic cell; MFI, mean fluorescence intensity; pDC, plasmacytoid DC

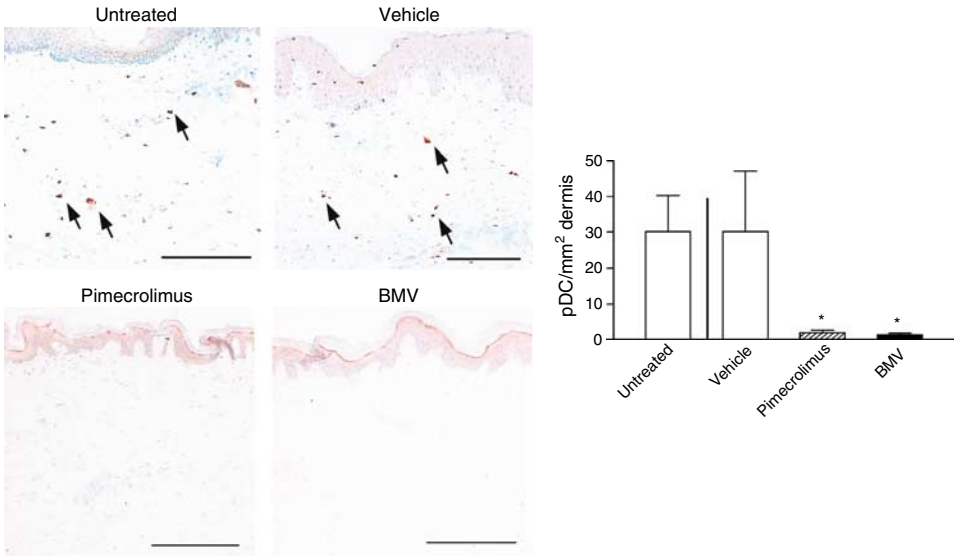

Figure 1. Topical treatment with pimecrolimus and BMV significantly depletes BDCA-2$^+$ cells in AD skin lesions. Cryostat sections (5 μm) were air-dried and fixed in ice-cold acetone for 10 minutes. To prevent nonspecific binding of the anti-BDCA-2 mAb (Miltenyi Biotec Inc., Auburn, CA), it was mixed with 1% normal horse serum and 2% BSA and applied to sections (1 hour, room temperature). Immunolocalization of the primary antibody was performed using a biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA), followed by an avidin-biotinylated-HRP third layer (Vector Laboratories). AEC + substrate (DakoCytomation, Glostrup, Denmark) were used as chromogen and sections were counterstained with Gill's hematoxylin (Santa Cruz Biotechnologies, Santa Cruz, CA). In all staining experiments, an IgG1 isotype mAb served as negative control. Representative pictures of immunostained sections were taken and positive cells are marked by arrows (upper panels). pDC numbers were evaluated in a blinded fashion (25–45 counted fields per section under $\times 25$ object lenses; numbers of patients per group: untreated = 22, vehicle control = 6, pimecrolimus = 8, BMV = 8) by two independent investigators and results are expressed as mean $\pm$ SEM positive cells/mm^2 dermis. One-way analysis of variance was used to evaluate statistically significant differences between test and control groups. *$P<0.05$ versus control. Bars = 200 μm.

the presence of plasmacytoid DC (pDC) has recently been demonstrated in the dermis and less abundant in the epidermis of AD lesions (Wollenberg *et al.*, 2002; Hashizume *et al.*, 2005). pDC are characterized by a plasma-cell-like morphology, a unique surface phenotype (HLA-DR$^+$CD1a$^-$CD123$^+$BDCA-2$^+$FcεRI$^+$), and the ability to produce large amounts of IFN-α, which assigns them a key role in innate antiviral immunity (Cella *et al.*, 1999; Siegal *et al.*, 1999).

In prior studies, we have investigated the impact and mode of action of CS and pimecrolimus on the DC population in murine and healthy human and lesional AD skin and have reported divergent effects of these compounds on Langerhans cell viability and function (Hoetzenecker *et al.*, 2004, 2005). In light of this finding, we asked whether these compounds also affect pDC. For this purpose, skin biopsies from a previous clinical trial were utilized (Hoetzenecker *et al.*, 2005). Briefly, 22 patients suffering from AD according to the diagnostic criteria of Hanifin and Rajka (Hanifin and Rajka, 1980; Rajka and Langeland, 1989) were assigned to treatment with 1% pimecrolimus cream (eight patients), matching vehicle cream (six patients), or 0.1% beta-methasone-17-valerate ((BMV), eight patients) in a 3-week, randomized, double-blind, vehicle-controlled parallel-group clinical trial. Creams were applied twice daily to all affected areas of the skin (except face) and punch biopsies (4 mm) were taken from acute lesions before treatment (day 0; begin of study) and on day 21 (end of study) after initiation of therapy. The study was conducted according to the guidelines of the Declaration of Helsinki Principles, approved by the local ethics committee of the Medical University of Vienna (code numbers: EK303/2003 and EK355/2005) and a written informed consent was obtained from all patients. Immunoperoxidase staining against the pDC-specific marker BDCA-2 revealed substantial pDC numbers in the dermis of untreated AD skin lesions (33 ± 8 cells/mm^2 dermis; $n = 22$), whereas pDC in normal healthy control skin were essentially absent (data not shown; $n = 5$). As shown in Figure 1, topical treatment with pimecrolimus and BMV for 21 days caused an almost complete disappearance of skin-infiltrating BDCA-2$^+$ cells by 94 and 95%, respectively, in comparison to vehicle-treated skin. The disappearance of BDCA-2$^+$ cells can be due to either the loss/down-regulation of this marker on the responder cell or due to actual deletion/destruction of pDC. In order to address this issue, pDC were isolated from peripheral blood of healthy adult volunteers (Figure 2a) and cultured with pimecrolimus and BMV at a concentration of 10^{-6} mol/l (Figure 2b). We have chosen this concentration intending to use the highest, non-toxic, biologically relevant dose for pimecrolimus to see an effect (Grassberger *et al.*, 1999; Kalthoff *et al.*, 2003). At selected time points, cells were analyzed for marker expression and viability. Neither pimecrolimus nor BMV downmodulated the expression of BDCA-2 on pDC at any time point investigated (Figure 2b).

However, we observed a marked reduction of BDCA-2$^+$ cells on BMV (92%), but not pimecrolimus (24%) culture already after 2 days when compared to vehicle (21%) (Figure 2c). After 7 days, pimecrolimus did not alter the viability of pDC when compared to vehicle (Figure 2c). In contrast, pDC on BMV culture were all dead at this time point (not shown). From these data, we conclude that the disappearance of BDCA-2$^+$ cells in AD lesions upon CS treatment most likely results from a depletion of pDC. The mechanism responsible for their depletion *in vivo* remains to be investigated. In keeping with recent reports from others (Shodell *et al.*, 2003; Suda *et al.*, 2003; Abe and Thomson, 2006), our data suggest that the reduction of pDC after topical treatment with CS may be owing to apoptosis. With regard to pimecrolimus, we have no evidence for direct effect on apoptosis in pDC in contrast to CS treatment. Theoretical possibilities are that indirect mechanisms such as cell migration and/or withdrawal of cytokines owing to an apoptosis-induced depletion of T cells upon pimecrolimus treatment (Hoetzenecker *et al.*, 2005) may be responsible for their depletion.

It is well known that patients with AD have recurrent bacterial or viral skin infections. The reason for this particular susceptibility is not entirely understood. Recent evidence exists that these individuals have a defect in the production of natural antimicrobial peptides (Ong *et al.*, 2002; Rieg *et al.*, 2005). Another reason could be a defect in other elements of the innate immune system. Indeed, pDC are less abundant in the infiltrate of AD lesions as compared to allergic contact eczema (Wollenberg *et al.*, 2002; Bangert *et al.*, 2003; Stary *et al.*, 2005). It will be interesting to see whether a functional defect of pDC may contribute to the high susceptibility of these patients to skin infections.

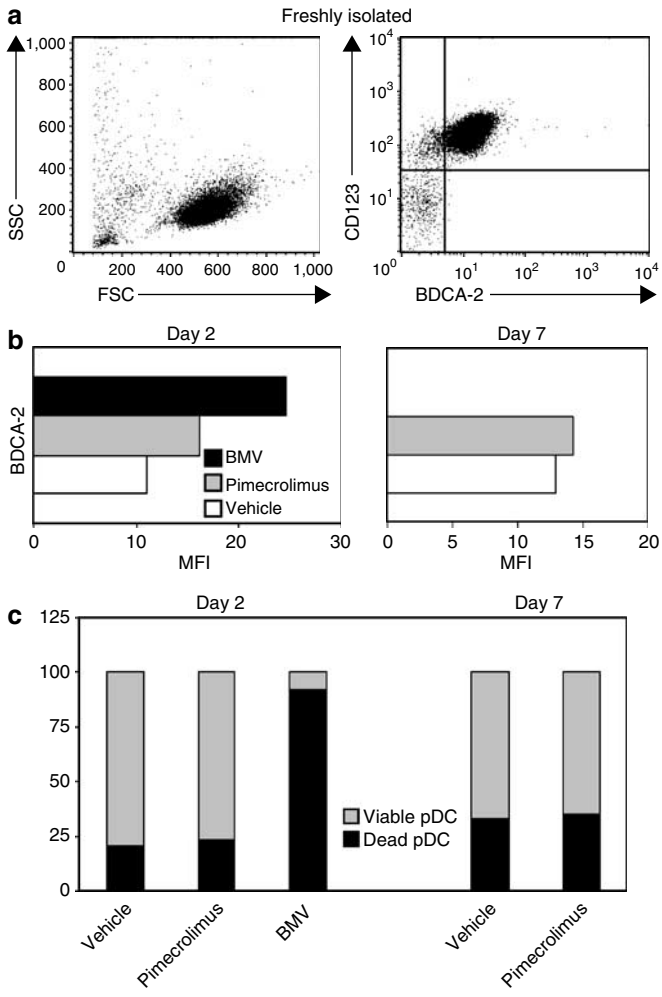

Figure 2. CS but not pimecrolimus strongly reduce pDC numbers *in vitro*. Buffy coats from peripheral blood of healthy adult volunteers were purchased from the local transfusion service (Rotes Kreuz, Vienna, Austria). Peripheral blood monocytes were isolated as interface cells after density gradient centrifugation (Lymphoprep; Axis-Shield, Rodelokka, Norway) and erythrocytes were removed with ammonium chloride (0.8% NH_4Cl/0.1 mM EDTA). pDC (mean purity: 93.88%±5.21 ($n=4$)) were isolated using a negative isolation kit according to the manufacturer's manual (Miltenyi Biotec Inc.). (**a**) Freshly isolated cells were stained for the indicated markers and 30,000 events/sample were acquired. pDC were cultured (5×10^6/ml) for the indicated time periods (day 2: $n=3$; day 7: $n=1$) in RPMI 1640 medium (GIBCO Life Technologies, Carlsbad, CA) supplemented with 10% FCS, 2 mM L-glutamine, antibiotics, and 10 ng/ml recombinant human IL-3 (Pepro Tech, Rocky Hill, NJ) in the presence of vehicle, pimecrolimus, or BMV (all 10^{-6} mol/l). Fluorescence was measured with a FACSCalibur flow cytometer, and data were analyzed using Cell Quest software (both Becton Dickinson, San Jose, CA). (**b**) Mean fluorescence intensity (MFI) was determined for BDCA-2 expression and (**c**) dead cells were identified by 7-amino-actinomycin D uptake (Sigma Chemicals Co., St Louis, MO).

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Drs G. Stary and C. Bangert for their kind and helpful advice. Financial support is acknowledged from Novartis Pharma AG, Basel.

Wolfram Hoetzenecker[1], Simone Meindl[1], Anton Stuetz[2], Georg Stingl[1] and Adelheid Elbe-Bürger[1]

[1]Department of Dermatology, Division of Immunology, Allergy and Infectious Diseases, Vienna Competence Center, Medical University of Vienna, Vienna, Austria and [2]Novartis Institutes for Biomedical Research, Vienna, Austria.
E-mail: adelheid.elbe-buerger@meduniwien.ac.at

REFERENCES

Abe M, Thomson AW (2006) Dexamethasone preferentially suppresses plasmacytoid dendritic cell differentiation and enhances their apoptotic death. *Clin Immunol* 118: 300–6

Bangert C, Friedl J, Stary G, Stingl G, Kopp T (2003) Immunopathologic features of allergic contact dermatitis in humans: participation of plasmacytoid dendritic cells in the pathogen-

esis of the disease? *J Invest Dermatol* 121: 1409–18

Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A *et al.* (1999) Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med* 5:919–23

Grassberger M, Baumruker T, Enz A, Hiestand P, Hultsch T, Kalthoff F *et al.* (1999) A novel anti-inflammatory drug, SDZ ASM 981, for the treatment of skin diseases: *in vitro* pharmacology. *Br J Dermatol* 141:264–73

Hanifin JM, Rajka G (1980) Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 92:44–7

Hashizume H, Horibe T, Yagi H, Seo N, Takigawa M (2005) Compartmental imbalance and aberrant immune function of blood $CD123^+$ (plasmacytoid) and $CD11c^+$ (myeloid) dendritic cells in atopic dermatitis. *J Immunol* 174:2396–403

Hoetzenecker W, Ecker R, Kopp T, Stuetz A, Stingl G, Elbe-Bürger A (2005) Pimecrolimus leads to an apoptosis-induced depletion of T cells but not Langerhans cells in patients with atopic dermatitis. *J Allergy Clin Immunol* 115:1276–83

Hoetzenecker W, Meingassner JG, Ecker R, Stingl G, Stuetz A, Elbe-Bürger A (2004) Corticosteroids but not pimecrolimus affect viability, maturation and immune function of murine epidermal Langerhans cells. *J Invest Dermatol* 122:673–84

Kalthoff ES, Chung J, Musser P, Stuetz A (2003) Pimecrolimus does not affect the differentiation, maturation and function of human monocyte-derived dendritic cells, in contrast to corticosteroids. *Clin Exp Immunol* 133: 350–9

Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA (2004) New insights into atopic dermatitis. *J Clin Invest* 113:651–7

Luger T, van Leent EJM, Graeber M, Hedgecock S, Thurston M, Kandra A *et al.* (2001) SDZ ASM 981: an emerging safe and effective treatment for atopic dermatitis. *Br J Dermatol* 144:788–94

Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T *et al.* (2002) Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 347:1151–60

Rajka G, Langeland T (1989) Grading of the severity of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 144:13–4

Rieg S, Steffen H, Seeber S, Humeny A, Kalbacher H, Dietz K *et al.* (2005) Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin *in vivo*. *J Immunol* 174:8003–10

Ruzicka T, Bieber T, Schöpf E *et al.* (1997) A short-term trial of tacrolimus ointment for atopic dermatitis. European Tacrolimus Multicenter Atopic Dermatitis Study Group. *N Engl J Med* 337:816–21

Shodell M, Shah K, Siegal FP (2003) Circulating human plasmacytoid dendritic cells are highly sensitive to corticosteroid administration. *Lupus* 12:222–30

Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S *et al.* (1999) The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284:1835–7

Stary G, Bangert C, Stingl G, Kopp T (2005) Dendritic cells in atopic dermatitis: expression of FcεRI on two distinct inflammation-associated subsets. *Int Arch Allergy Immunol* 138:278–90

Stoppolino G, Prisco F, Santinelli R, Sicuranza G, Giordano C (1983) Potential hazards of topical steroid therapy. *Am J Dis Child* 137:1130–1

Suda T, Chida K, Matsuda H, Hashizume H, Ide K, Yokomura K *et al.* (2003) High-dose intravenous glucocorticoid therapy abrogates circulating dendritic cells. *J Allergy Clin Immunol* 112:1237–9

Wollenberg A, Wagner M, Günther S, Towarowski A, Tuma E, Moderer M *et al.* (2002) Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases. *J Invest Dermatol* 119:1096–102